A CHECKLIST OF FORENSICALLY IMPORTANT INSECT TAXA ON DECOMPOSING CARCASSES AND SUCCESSIONAL PATTERNS RELATED TO DECOMPOSITION AND SEASON IN MINNESOTA

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DEDICATION

My thesis work is dedicated to Trent Reznor. Oh! And my husband.

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ABSTRACT

Numerous studies in North America have focused on the entomofauna associated with decomposing pig carcasses. However, research is lacking in the upper Midwest region of the United States. This study establishes a reference checklist of forensically important insect taxa throughout stages of decomposition of a carcass and investigates these taxa in relation to both stage of decomposition and season in Minnesota. Eleven pig carcasses (*Sus scrofa)* were placed in the field at monthly intervals from May 2017 through September 2018 and were examined daily to collect both immature and adult insects. Collections were with aerial net, pitfall traps, and hand collection. Adult specimens were identified and preserved. A portion of calliphorid larvae were also reared to adult in the laboratory for identification and preservation. In total, 64 forensically important insect taxa were recorded, representing 14 families, and including 12 species of Calliphoridae. *Phormia regina* and *Lucilia sericata* were consistently abundant through temperate seasons. Calliphoridae dominated the early stages of decomposition, and Coleoptera increased in the later stages of decomposition. The results of this study will serve as a practical tool of geographic reference of forensically important insects for local law enforcement agencies and forensic entomologists investigating cases of unnatural death in Minnesota, and also will serve as a foundation for further succession research in the region.

Chapter 1: Introduction and Literature Review

1.1 Introduction

The close association between necrophagous insects and decomposition of human tissues provides a useful tool in death investigation. Insects use a decomposing body as an ephemeral resource for food and development (Hall, 2001). The succession of necrophagous insects throughout the stages of decomposition provides information that forensic entomologists (FEs) analyze to estimate a time of death. The analysis of this evidence can also answer questions surrounding a death, aiding the criminal justice system.

When examined by a forensic entomologist, insect evidence can be used to answer several questions. Often, an antemortem injury, or injury occurring prior to or during death, can result in concentrated insect activity at the site of injury (Hall, 2001). This can often help answer the question of *how* death occurred. The presence or absence of insect communities can indicate the movement of a body postmortem, lending a clue to the question of *where* death took place. And often the most important question that insect evidence can answer is: *when* did death occur?

Blow flies (Diptera: Calliphoridae) will visit a decomposing body within minutes of death (Smith, 1986), making them reliable resources that offer information aiding in a death investigation. The succession of insects throughout the predictable stages of decomposition, and the temperature-dependent development of certain necrophagous insect species, allow forensic entomologists to construct an accurate timeline in cases of unnatural death. This timeline is referred to as the post-colonization interval (PCI), or the time between initial colonization by insects and the time when human remains were discovered (Hall, 2001).

Today, FEs assist in legal and criminal cases involving insect evidence using the wealth of research available within forensic entomology. The temperature-dependent development of the first calliphorid larvae to colonize a body allow FEs to model development time and estimate the minimum amount of time since colonization.

The necrophagous insects that can be used as evidence make up a long list of taxa that can vary by the geographic range in which they are found. For this reason, a reference list of the local forensically important insect fauna is a useful tool to aid in a death investigation. The ability to match collected insect evidence with what is expected to occur in the region would be incredibly beneficial. There is existing insect succession research dotted across the continent; however, the useful information that an initial study of insect succession throughout the stages of decomposition would provide is lacking in the upper Midwest of North America. This thesis describes research to provide a necessary reference list of insect taxa for the upper Midwest and describes the successional patterns related to decomposition throughout the seasons of Minnesota.

1.2 Defining Forensic Entomology

Forensic entomology is an umbrella term used to describe any intersection of insects and legal matters. There are three defined studies of insects that fall within the realm of forensic entomology. Each are related in that they involve infestations and typically intersect with legal repercussions. Stored product entomology involves insect infestations of food products (Hall, 2001). Urban entomology has traditionally been considered to involve insect infestations of manmade structures, but this definition has expanded to include insect infestations of human environments (Hall, 2001). Medicolegal entomology concerns the use of necrophagous insects to aid in the investigation of unnatural death, and sometimes cases of abuse or neglect of humans and animals (Hall, 2001). For the purpose of this thesis and simplicity, the term forensic entomology will be used in the context of medicolegal entomology.

1.3 A Brief History of Forensic Entomology

Dating back to the thirteenth century, forensic entomology provided answers to questions that involved wrongful death. In 1235, the Chinese death investigator Sung Tz'u penned *The Washing Away of Wrongs* in which he detailed an instance of early forensic entomology solving a homicide in a farming village (McKnight, 1981). Following the violent death of a villager, an investigator questioned the entire community. Unable to conclude the case, the investigator then requested that each villager stand in a line and place their farming implements on the ground in front of them. Attracted by the traces of blood, flies gathered on only one sickle; faced with this evidence, the owner confessed to slashing the victim to death.

A significant step in forensic entomology came four centuries later when Italian physician Francesco Redi disputed and disproved the widely accepted idea that maggots spontaneously generated from rotting meat (Hall, 2001). In 1668, Redi used rotting meat to attract flies to show that maggots emerged from eggs the flies laid as part of their reproductive cycle, rather than simply appearing on the putrefying substrate. This experiment was one of the first to show that insects possessed a complex life cycle (Buettner, 2007).

In 1855, Paris, France, the unearthing of a partially mummified infant within the walls of a dwelling launched an investigation of the family residing in the home at the time of discovery. Louis Etienne Bergeret used the idea of insect succession on a cadaver to propose that the corpse was several years old. This conclusion provided evidence of the innocence of the current tenants, making Bergeret the first westerner to use insect evidence forensically (Hall, 2001).

Again, in France, Jean-Pierre Mégnin provided another milestone with the publication of several articles encouraging the use of insect evidence in the aid of investigations. *La Faune des Cadavres: Application l'entomologie a la Medicine Legale* (Hall, 2001; Mégnin, 1894) is considered by many in the realm of forensic entomology to be one of the most important texts in this field of study. Mégnin related insect activity to stages and duration of decomposition, lending to the use of arthropods in legal and criminal investigation (Gennard, 2007).

Two 20th century publications have been the most influential in furthering research within the field of forensic entomology. David Hall published *The Blowflies of North America* in 1948, making available detailed descriptions of both calliphorid adults and 3rd-instar calliphorid larvae (Hall, 2001). Decades later in 1986, Kenneth Smith published *A Manual of Forensic Entomology,* widely considered to be the first textbook devoted solely to the subject (Hall, 2001).

1.4 Forensic Entomology Today

An estimate of time of death can be offered by a forensic pathologist or medical examiner based on several procedures such as measures of potassium levels in the vitreous humour, rigor mortis, or body temperature (Estracanholli *et al.*, 2009). However, these techniques are limited in several ways. They are either subjective and lack quantitative measure, or are quantifiable, but limited by the window of time in which they are measurable (Estracanholli *et al.*, 2009; Zilg *et al.*, 2015). Conclusions about time of death based on these procedures can be supported, or possibly refuted with insect evidence collected at the scene of death.

Insect evidence can be used to estimate a time of death in two manners: (1) by creating a reverse timeline for development of the oldest immature stages of blow flies collected at the scene of death, and (2) by determining the length of time since colonization based on a characteristic wave in insect communities that develops throughout the stages of decomposition (Gennard, 2007; Goff, 1993). Approaching the minimum time of death estimation through larval development is appropriate to estimate post-colonization interval for shorter lengths of time following death, up to a month postmortem (Smith, 1986). Estimating a post-colonization interval through insect succession is appropriate when investigating longer intervals of time following death (Smith, 1986). This characteristic succession of insects observed throughout the stages of decomposition is directly related to the ecological roles of these forensically important insects and their ability to drive physical decomposition.

1.5 Human Decomposition

Within minutes of death, processes inside a body start the chemical breakdown of cells and tissues (Vass, 2001). Human decay is caused and continued by two types of decomposition: chemical and physical (Perper, 2004). Products of chemical decomposition are what make it attractive to insects. Physical decomposition is driven by the consumption of tissues by insects.

Chemical decomposition occurs first by autolysis, or the breakdown of cells by enzymes, and later by putrefaction, the further degradation of body tissues by micro-bacterial activity (Li *et al.*, 2016; Perper, 2004; Vass, 2001). This tissue breakdown is responsible for the release of gases that contribute to the bloating of a decomposing body, and additionally, attracts the first calliphorid adults to the body. These flies will visit a decomposing body within minutes, often from miles away (Carter *et al.*, 2007; Smith, 1986). Due to the unlikelihood of visual detection, research has shown that volatile organic compounds (VOCs) released during decay are attracting insects via semiochemicals (Frederickx *et* *al.*, 2012), the chemical cues to which an insect responds. The molecules of a VOC stimulate the sensitive chemoreceptors of an insect, allowing it to detect a decomposing body in the absence of a visual cue (Frederickx *et al.*, 2012). Additionally, attraction to a body is accelerated by intraspecific pheromones released by initial colonizers that stimulate oviposition by conspecifics (Frederickx *et al.*, 2012).

The insects that are attracted to decay via VOCs are largely responsible for the physical decomposition of a body (Carter *et al.*, 2007). Initial colonizers, typically calliphorid adults, will visit a body to feed, mate, and oviposit (Smith, 1986). Most Calliphoridae are anautogenous, meaning a protein meal is required to produce eggs (Rivers & Dahlem, 2013; Webber, 1958), and they will use body fluids as the protein meal. Gravid adult Calliphoridae deposit clutches of eggs in moist body openings, often the eyes, nasal mucous membranes, and mouth (Goff, 2009; Rivers *et al.*, 2011). The larvae that emerge from these eggs, along with other necrophagous insects, will break down the tissues of a body, beginning a succession of insects that progresses a body through ordered stages of decomposition.

Carcasses from a wide range of animal species have been used to study insect succession and decomposition; however, pig (*Sus scrofa* L.) carcasses are thought to be the most appropriate substitute for humans. The tissues of swine carcasses closely resemble those of humans, making the physical decay of the epidermis similar to that of a body (Goff, 1993). Pig carcasses are also large enough to dismiss the idea that model size may affect rates of decomposition (Payne, 1965). Additionally, the gut fauna of a pig is similar to that found in humans (Goff, 1993). Lastly, the availability of pig carcasses, rather than relying on roadkill, makes for a consistency of decomposition models (Goff, 1993).

The first insect succession study using pig carcasses described six stages of decomposition (Payne, 1965), and the same classification system was adopted by other investigators (Carter *et al.*, 2007). More recently, researchers have condensed the six stages into five, with the following recognizable characteristics (Goff, 1993; Goff, 2009).

The first stage of decomposition is the “fresh” stage and begins at the moment of death (Galloway, 1989; Goff, 1993; Goff, 2009). Tissues appear unaffected by chemical processes occurring within the body. However, it is at this stage that the first VOCs are released, attracting adult calliphorids that colonize a body. Calliphorid eggs are laid in open orifices and first instar larvae hatch from these eggs and begin to consume tissues. This first stage of decomposition ends when the body begins to fill with gases and appears to be inflated (Goff, 1993; Goff, 2009).

The second stage of decomposition, “bloat”, occurs when a body begins to inflate from gases released from microbial putrefaction within tissues (Galloway, 1989; Goff, 1993; Goff, 2009). Skin discoloration occurs and marbling becomes evident, as well as blistering and slippage of the epidermis (Goff, 1993; Goff, 2009; Perper, 2004). Skin rupture eventually occurs, typically in the abdomen, allowing maggots to access internal abdominal tissues (Carter *et al.*, 2007). The increased larval activity on these exposed tissues leads into the next stage of decomposition.

The third stage of decomposition is "active decay" (Carter *et al.*, 2007) or just "decay" (Goff, 1993; Goff, 2009), signified by the greatest amount of tissue loss, largely due to aggregations of maggot activity. During this stage, fluids are evident around the body, seeping into the surrounding soil and vegetation if outdoors (Carter *et al.*, 2007). Predaceous insects will be more abundant as their food source, calliphorid larvae, becomes plentiful (Goff, 2009). The end of active decay occurs when calliphorid larvae disperse away from the body to pupate and complete the rest of their life cycle (Goff, 2009).

The fourth stage of decomposition is "advanced decay" (Carter *et al.*, 2007), or "postdecay" (Goff, 1993; Goff, 2009). This stage is characterized by the absence of calliphorid activity. All that is left behind is mostly bones, connective tissue, and skin (Goff, 1993; Goff, 2009). Calliphoridae activity, both adult and larval, decreases as the body no longer possesses the necessary substrates for feeding, oviposition, or development. However, their absence is filled with the activity of various Coleoptera, mainly dermestid larvae and adults (Goff, 1993; Goff, 2009).

The last stage of decomposition is "dry remains" or "skeletal/remains", where not much besides bones and leathery skin remain at this stage in which dermestid larvae and adults are present (Goff, 1993).

1.6 Biology of Calliphoridae

Decomposition involves successive waves of insects, which begin with Diptera. Calliphoridae and Sarcophagidae are the first to arrive at a body and use the decomposing tissues to complete their life cycles (Greenberg, 1991). Calliphoridae are the most abundant on a decomposing body and are important for establishing a time of death.

Calliphoridae is a large family of Diptera, comprised of over 1,000 species, with 54 species found in the United States and Canada (Whitworth, 2006). Adults are medium-sized calyptrate flies ranging from 6 to 14 mm in length (Byrd & Castner, 2001). They are most often metallic in appearance and commonly display brilliant colors ranging from dark greenish-black, vivid coppery-green or bluish-green, to dull-ish dark blue (Byrd & Castner, 2001; Hall, 1948; Whitworth, 2006). Larvae are a cream-white color (Byrd & Castner, 2001), and like all other muscoid dipteran larvae, are unsclerotized save for the cephalopharyngeal skeleton, setae and posterior spiracular plates (Torre-Bueno, 1871-1948 (1989)).

Calliphoridae exhibit a holometabolous life cycle with stages of egg, larva, pupa, and adult. Calliphorid eggs are laid in clusters and they are cream-white and cylindrical. After hatching from the egg, the larva goes through three instars. Instar can be determined by the number of slits found on the posterior spiracular plate; first instar larvae have one slit, second instar larvae have two slits, and third instar larvae have three slits (Byrd & Castner, 2001). Third instar larvae range from 8 to 23 mm in length (Byrd & Castner, 2001). The third instar larva will evacuate its gut as it vacates its feeding substrate in preparation for pupation.

Wandering post-feeding larvae will find concealed locations to form puparia where they complete metamorphosis and eventually emerge as an adult blow fly. The puparium will appear football-shaped and darken in color with time to a reddish-brown (Smith, 1986).

1.7 Insect Succession

Forensic entomology literature generally recognizes four functional groups of insects that occur on decomposing cadavers based on ecological niche: 1) necrophagous insects, 2) predators and parasitoids, 3) omnivorous insects, and 4) incidental insects (Catts & Goff, 1992; Goff, 1993; Smith, 1986). The first three groups make up the taxa considered to be forensically important (Arnaldos *et al.*, 2005; Goff, 1993), and for simplicity, this research focuses on these three groups. These groups and their ecological niches make up the characteristic successive pattern of insects that are observed throughout the stages of insects. Special focus is given to the family Calliphoridae, as they are the initial colonizers of a decomposing body that offer the most accurate estimate of death in legal investigations.

Necrophagous insects use a cadaver as a food source (Catts & Goff, 1992; Goff, 1993; Smith, 1986). This group is typically comprised of Calliphoridae, Sarcophagidae, and Dermestidae (Goff, 1993; Smith, 1986). Calliphorid adults and larvae actively feed on the tissues or imbibe fluids throughout the fresh, bloat, and active decay stages of decomposition, whereas dermestid adults and larvae are typically present at advanced decay and dry remains stages of decomposition when only skin and bones are left.

Predators and parasitoids can occur throughout the stages of decomposition whenever their prey is present. Species of Staphylinidae and Histeridae will appear as soon as calliphorid maggots are present (Smith, 1986). Parasitoids of fly pupae, such as braconid wasps, are also included in this group (Catts & Goff, 1992; Goff, 1993).

Omnivorous insects include those that consume cadaver tissues, as well as the necrophagous insects that are present (Arnaldos *et al.*, 2005; Catts & Goff, 1992; Goff, 1993; Smith, 1986). Omnivores at carcasses include vespid wasp adults, silphid larvae and adults, and clerid adults. Vespid wasps (Hymenoptera) will imbibe fluids that seep from a cadaver; they also prey on adult Diptera aggregated at a body (Smith, 1986). Similarly, silphid larvae and adults (Coleoptera) will take advantage of both the decomposing organism and the dipteran larvae, resulting in their presence mainly through the active and early parts of the advanced stages of decomposition (Smith, 1986). Clerid beetles (Coleoptera) are found across many stages of decomposition, as they seek out both dipteran larvae and dermestid larvae, as well as the decomposing matter on which these larvae are found (Smith, 1986).

Ecological relationships for some insects present on carcasses are unclear. While Nitidulidae (Coleoptera) may be present at cadavers to feed on the decomposing tissues, Smith (1986) implies that feeding habits are not known with certainty and literature about which ecological role the beetles fill is hard to find. While Byrd and Castner (2001) do not address what nitidulid adults are doing at a cadaver, they do detail that *Omosita colon* (L.) of Nitidulidae is found in the later stages of decomposition and seems to prefer moist environments.

1.8 Faunal Surveys and Succession Studies

Studies performed throughout North America have examined insect succession throughout the stages of decomposition of animal carcasses (Table 1.1). Many recent studies are a continuation of initial research, assessing insect succession and decomposition as influenced by different variables.

The first study of insect succession was carried out in South Carolina, where Payne (1965) compared the decomposition and insect succession in carcasses exposed to insects with carcasses kept free from insects. Payne's research set the standard for use of pigs as models for human cadavers.

Insect succession on decomposing carcasses has been recorded in Hawaii, British Columbia, Colorado, Michigan, Ohio, Louisiana, Mississippi, Virginia, North Carolina, and Florida (Anderson & Vanlaerhoven, 1996; Benbow *et al.*, 2013; Cruise *et al.*, 2018; DeJong, 1994; Early & Goff, 1986; Goddard *et al.*, 2012; Gruner *et al.*, 2007; Pastula & Merritt, 2013; Tabor *et al.*, 2004; Watson & Carlton, 2003). Table 1.1 shows existing succession research by state or province and highlights Calliphoridae found. The table is organized by geographic location from west to east and highlights the range of these calliphorid species. The total column on the right displays the number of studies in which each calliphorid species has been recorded; the total row at the bottom of the table displays the number of calliphorid taxa each study recorded. Table 1.1 reflects data from a single study in each reported state or province.

Table 1.1 Calliphoridae collected on decomposing carcasses throughout North America by region.

		West			Central		South					
Subfamily	Species	HI	BC	CO	MI	OH	LA	MS	VA	NC	FL	Total
Calliphorinae	*Calliphora alaskensis*	-	-	+	-	-	-	-	-	-	-	1
	Calliphora coloradensis	-	-	+	-	-	-	-	-	-	-	1
	Calliphora latifrons	-	+	-	-	-	-	-	-	-	-	1
	Calliphora lilaea	-	-	+	-	-	-	-	-	-	-	1
	Calliphora livida	-	-	+	-	-	-	-	-	-	+	2
	Calliphora terraenovae	-	-	+	-	-	-	-	-	-	-	1
	Calliphora vicina	-	+	+	-	-	-	-	+	-	+	4
	Calliphora vomitoria	-	-	+	-	-	-	-	+	-	-	2
	Cynomya cadaverina	-	+	+	-	-	-	-	-	-	-	2
Chrysomyinae	*Chrysomya megacephala*	+	-	-	-	-	-	-	-	-	+	2
	Chrysomya rufifacies	+	-	-	-	-	+	-	-	-	+	3
	Cochliomyia macellaria	-	-	+	-	+	+	+	-	+	+	6
	Phormia regina	-	+	+	+	+	+	+	+	+	+	9
	Protophormia terraenovae	-	+	+	-	-	-	-	-	-	-	2
Luciliinae	*Lucilia coereuliviridis*	-	-	-	+	+	+	+	+	+	+	7
	Lucilia cuprina	+	-	-	-	-	+	+	-	+	-	4
	Lucilia illustris	-	+	+	+	+	-	-	+	+	-	6
	Lucilia sericata	-	+	+	-	+	+	-	+	+	-	6
	Total:	3	7	13	3	5	6	4	6	6	7	

Succession studies began in Oahu, Hawaii in the 1980’s, where insect succession was observed and recorded with detail given to the stages of decomposition (Early & Goff, 1986). Early and Goff (1986) found that the difference in insect communities at two sites resulted in differing duration of decomposition stages.

The closest study geographically to Minnesota was in Clear Creek County, Colorado, where insect fauna were recorded on rabbit carrion at a high elevation site (DeJong & Chadwick, 1999). DeJong and Chadwick (1999) reported thirteen calliphorid species among 53 insect taxa.

The closest study geographically to the east was conducted in East Lansing, Michigan, where insect succession was examined on buried carrion (Pastula & Merritt, 2013). This study recorded 3 calliphorid species along with other muscid species and various Coleoptera.

Degree days were applied to stages of decomposition as community composition diversity was examined in Ohio (Benbow *et al.*, 2013). The study focused on abundance for five taxa, noting a significant difference in abundance across seasons; however, it was reported that the composition of the insect community observed was generally consistent (Benbow *et al.*, 2013).

In Louisiana, various wildlife carcasses were used to investigate whether there was variation in the visiting insect fauna, addressing questions regarding poaching (Watson & Carlton, 2003). This study noted similarities of insect succession on fur-bearing carcasses, but significant variation of insect succession on an alligator carcass (Watson & Carlton, 2003).

The findings from a class in Mississippi project were published in which insect succession was examined (Goddard *et al.*, 2012). This study found *Phormia regina* (Meigen) to be most abundant out of 4 calliphorid species collected (Goddard *et al.*, 2012).

Succession was qualitatively assessed in southwestern Virginia across various seasons; reserachers found that a greater number of insect taxa visited carcasses in spring when compared to summer (Tabor *et al.*, 2005). Calliphorid adults were represented by eight species during the two-year study (Tabor *et al.*, 2004).

All insect taxa were recorded through decomposition in late summer in North Carolina (Cruise *et al.*, 2018). Cruise *et al.* (2018) reported six Calliphoridae species, as well as various Coleoptera taxa. This study examined the succession of these insects throughout the stages of decomposition, and observed that the patterns of arrival were as expected and followed the order of ecological roles (Cruise *et al.*, 2018).

In Florida, species distribution of Calliphoridae was assessed through all seasons of the year (Gruner *et al.*, 2007). Gruner *et al.* (2007) recorded seven species of calliphorid adults with *Lucilia coeruleiviridis* Macquart being the most abundant.

Common, widespread calliphorid species, such as *Cochliomyia macellaria* (F.), *Lucilia illustris* (Meigen) and *L. sericata* (Meigen), are each recorded in 7 studies situated quite evenly across North America, and *Phormia regina* even more so, found in all studies with the exception of Hawaii (Table 1.1). Additionally, *Calliphora vicina* Robineau-Desvoidy, *C. vomitoria* (L.), and *Cynomya cadaverina* Robineau-Desvoidy are common and widespread but recorded in far fewer studies (Table 1.1). Some calliphorid species are widespread, but rare, such as *Calliphora terraenovae* Macquart, only recorded in Colorado (DeJong & Chadwick, 1999) (Table 1.1).

The calliphorid species recorded across the continent in Table 1.1 highlight the geographic patterns that range plays in species presence. Species whose range includes the northern United States, Canada and Alaska, such as *Protophormia terraenovae* (Robineau-Desvoidy) (Whitworth, 2006) are recorded in research in Vancouver (Anderson & Vanlaerhoven, 1996), and Colorado (DeJong & Chadwick, 1999), as expected. Species primarily found in the southern range of the continent, such as *Chrysomya rufifacies* (Macquart) (Whitworth, 2006) are recorded in research in Hawaii (Goff *et al.*, 1986), Louisiana (Watson & Carlton, 2003) and Florida (Gruner *et al.*, 2007). Record of these species within their geographic range is expected; yet, it highlights the need for a more complete picture of what occurs in areas of geographic overlap.

This study aims to investigate what forensically important insect taxa visit decomposing carcasses in Minnesota, with a focus on calliphorid species. Previous research described above would suggest a geographic overlap of calliphorid species. Regarding Calliphoridae, I expected to find an abundance of widespread species found across the continent and a natural overlap of species whose geographic range runs close or through Minnesota. For instance, I expected to find *Phormia regina*, whose abundance is evident with record of this species in every study with the exception of Hawaii (Table 1.1). I also expected to see a number of calliphorid species that aligned with records out of the closest studies to the west and east, Colorado and Michigan. However, Colorado reported thirteen calliphorid taxa, while Michigan recorded only 3 (DeJong & Chadwick, 1999; Pastula & Merritt, 2013). This is mostly like because the work out of Michigan was an incomprehensive study that examined the effects of burial on carrion; while it provides insect presence data, the nature of the study may have limited the visiting fauna. Due to this limited data, it is difficult to predict what we may see here in Minnesota due to proximity and geographic overlap of species.

The significance of this research is that it is the first study of insect succession on a decomposing body in the upper Midwest, laying the necessary groundwork for future research here, and supplying a necessary tool for local and regional professionals in entomology and law enforcement. This research can be compared to succession research throughout North America, as summarized by Table 1.1.

My research had two objectives:

1. to build a reference checklist of forensically important taxa that occur in Minnesota, with a focus on species of Calliphoridae.
2. to detail the successional patterns related to decomposition throughout the seasons of Minnesota in regard to forensically important insect taxa.

Thorough emphasis is given to Calliphoridae. While all other taxa were collected and analyzed, the results and discussion of each objective focuses mostly on the species of Calliphoridae. Specimens of remaining families of Diptera and Coleoptera were identified to genus, at minimum, and have been grouped to family in the following sections.

Chapter II: Checklist of forensically important insect taxa on decomposing pig carcasses in Minnesota

2.1 Introduction

The initial dipteran colonizers and other insect taxa that occur throughout the stages of decomposition of a human body provide useful information to a FE, as outlined in Chapter I. Depending on a body's stage of decomposition, a qualified expert can use either the temperature-dependent development of the initial colonizers, most often Calliphoridae, or the succession of insects that occurs throughout decay to determine time of death. Insect succession research has been conducted through various regions of North America; however, insect species that occur on a decomposing body can differ by geographic range (Table 1.1). the objective of this chapter is to assemble a checklist of forensically important insect taxa that occur on decomposing bodies in the upper Midwest. The checklist will provide a foundation for future research in forensic entomology in the upper Midwest.

2.2 Materials and Methods

For this research, I sampled pig carcasses during the growing season from spring 2017 to fall 2018 for associated insect larvae, pupae, and adults. Pig carcasses were laid out once a month. In 2017, the October carcass was the last carcass laid out due to colder temperatures that halted insect activity and decomposition. This carcass was left out during the winter and sampling resumed in the spring. The last carcass laid out in 2018 was the September carcass.

2.2.1 Research Site and Study Plot

The University of Minnesota Dairy Cattle and Research Barn, located in St. Paul, MN (44.985596°W, 93.175886°N) hosted this research. The northeast corner of an area housing calf hutches was cleared of debris, then weeded and tilled to provide a semi-bare ground surface for a study plot. There was a cow barn to the north, pastures to the east and calf hutches to the west. Any biological waste produced from the dairy barn was disposed of by University of Minnesota Animal Waste Removal procedures, eliminating possible outside sources of forensically important insects.

A raiseable platform for the carcasses was constructed with zinc-plated angle iron frame and 1” chicken wire in order to have access to the underside of the carcass. The 1” mesh of the chicken wire allowed the carcasses to be in contact with the ground. A cage to exclude scavenging vertebrates was made of PVC pipe and 1” x 1.5” deer fencing. The cage was attached to the platform with flexible gear ties, and pitfall traps containing an antifreeze solution were placed at each corner of the platform to catch ground-dwelling insects.

A HOBO data logger (Onset: Bourne, MA) was erected at standard meteorological service height of 1.5 m near the carcass to record temperatures from four microhabitats: ambient air temperature, soil temperature 1 cm.

underneath the abdomen of the carcass, and one each in the pharynx and rectum of the carcass to record internal temperatures.

2.2.2 Carcasses

Male pigs initially weighing approximately 27 kg were euthanized with a captive blitz bolt at Manthei Pig Farm at 23130 112th St. NW, Elk River, MN. The University of Minnesota Institutional Animal Care and Use Committee was consulted, and they declared that animal-use guidelines were not applicable because the study did not involve living animals. Carcasses were immediately placed in a coroner's body bag to prevent insect colonization prior to placement at the research site. Transport from the pig farm to the research plot was approximately 64 km.

2.2.3 Carcass sampling

The first carcass was placed at the research plot on 8 May 2017 (Table 2.1); subsequent carcasses were placed at monthly intervals thereafter depending on weather and alignment of carcass supplier schedules. Day of each placement was recorded as day 0. I recorded weather observations and decomposition observations daily. Each day thereafter, I collected adult insects by aerial netting; I collected adults and larvae on, underneath, and inside the carcass with forceps or by hand; and I used pitfall traps to collect ground-inhabiting adults and larvae. This routine continued until advanced decay, at which time I moved carcass remnants to a smaller platform at a new plot at a minimum distance of 50 feet from the original plot, and sampling frequency was reduced to once a week due to decreased insect activity.

Table 2.1 Dates of carcass placement. University of Minnesota campus dairy, spring 2017 to fall 2018.

	Carcass trial	Date of placement
2017	May	8 May
	June	9 June
	July	15 July
	August	7 August
	September	8 September
	October	12 October
2018	May	27 April
	June	4 June
	July	9 July
	August	1 August
	September	14 September

Some methods were altered in 2018 due to the focus on Calliphoridae: carcasses were placed directly on the bare research plot rather than a raiseable platform and were disposed of once they reached the advanced decay stage.

2.2.4 Specimen Identification and Preservation

I pinned and labeled adult specimens obtained by netting and rearing, and a fraction of larval specimens were preserved in 80% ethanol. I collected a portion of the blow fly larvae live and reared these larvae to adult in the lab for ease of identification. These larvae were raised on beef liver and incubated at 25°C.

I identified adult specimens of Diptera using taxonomic keys (Peterson *et al.*, 1981, 1981; Whitworth, 2006). Verification for a portion of calliphorid adults were confirmed by Dr. Terry Whitworth of Washington State University. Additional verification of non-calliphorid Diptera and Coleoptera was provided by Drs. Roger Blahnik, Ralph Holzenthal, and John Luhman of the University of Minnesota Insect Collection.

Voucher specimens will be deposited in the University of Minnesota Insect Collection.

2.3 Results & Discussion

Overall, I identified and preserved 64 taxa representing three insect orders (Table 2.2). These specimens represent insect taxa across various life stages and are shown by insect order. All tables reflect the life stage at which the specimen was collected. Of those specimens, 67% were Diptera, 29% were Coleoptera, and 4% were Hymenoptera. Previous research with a forensic focus yielded similar proportions, with as few as 16 and as many as 80 identified taxa recorded (Anderson & Vanlaerhoven, 1996; Benbow *et al.*, 2013; Cruise *et al.*, 2018; Tabor *et al.*, 2004).

Six families of Diptera were considered forensically important: Calliphoridae, Sphaeroceridae, Piophilidae, Muscidae, Sarcophagidae, and Sepsidae.

Calliphoridae was the most diverse family with 12 species identified (Table 2.2), representing three subfamilies: Calliphorinae, Chrysomyinae, and Luciliinae. Within Calliphorinae, five species were identified; within Chrysomyinae, three species were identified; within Luciliianae, four species were identified. The majority of reared-to-adult calliphorid larvae were *Phormia regina* and *Lucilia sericata*; however, *Calliphora vicina*, *Cochliomyia macellaria*, *L. coeruleiviridis*, and *L. illustris* were also collected as larvae and lab-reared to adult though less frequently and more sporadically. (Table 2.3).

I identified remaining Diptera to genus, and to species where possible (Table 2.2).

Within Coleoptera, seven families of forensically important species were obtained (Table 2.2): Staphylinidae, Silphidae, Dermestidae, Histeridae, Cleridae, Scarabaeidae, and Nitidulidae. Only two Hymenoptera were collected as forensically important insect taxa, both identified to family level: Braconidae and Vespidae (Table 2.2).

2.3.1 Calliphoridae

Within Diptera, Calliphoridae was the most diverse in species. With 12 taxa identified (Table 2.2), Calliphoridae represented 32% of Diptera and 22% of all insects identified. These percentages are lower than previous research which reported that Calliphoridae made up 60% of total Diptera collected (Tabor *et al.*, 2004); however, diversity within the family was found to be lower (8 species) in the Virginia study (Tabor *et al.*, 2004). Species were identified from three subfamilies: Calliphorinae, Chrysomyinae, and Luciliinae.

Calliphoridae collected and identified represent forensically important species found in the upper Midwest. Those collected onsite as adults only include *Calliphora latifrons* Hough, *Ca. terraenovae*, *Ca. vomitoria*, *Cynomya cadaverina*, *Protophormia terraenovae*, and *Lucilia silvarum* (Meigen). *Calliphora latifrons*, *Ca. terraenovae*, *Ca. vicina*, and *Ca. vomitoria* were found only sporadically. Ambient temperatures at the time of these collections reflect the cold-hardiness of the genus (Faucherre *et al.*, 1999). Each *Calliphora* species

Table 2.2 Sixty-four forensically important insect taxa collected on 11 decomposing pig carcasses. University of Minnesota St. Paul campus dairy, spring 2017 to fall 2018.

Order	Family	Genus/Species	Life Stage
	Calliphoridae	*Calliphora latifrons* Hough	A*
		Calliphora terraenovae Macquart	A
		Calliphora vicina Robineau-Desvoidy	A, I*
		Calliphora vomitoria (Linnaeus)	A
		Cynomya cadaverina Robineau-Desvoidy	A
		Cochliomyia macellaria (Fabricius)	A, I
		Phormia regina (Meigen)	A, I
		Protophormia terraenovae (Robineau-Desvoidy)	A
		Lucilia coeruleiviridis Macquart	A, I
		Lucilia illustris (Meigen)	A, I
		Lucilia sericata (Meigen)	A, I
		Lucilia silvarum (Meigen)	A
Diptera	Muscidae	*Azelia* spp. Robineau-Desvoidy	A
		Hydrotaea spp. Robineau-Desvoidy	A
		Morellia spp. Robineau-Desvoidy	A
		Musca spp. Linnaeus	A
		Thricops spp. Ródani	A
		Muscidae spp.	A
	Piophilidae	*Allopiophila* spp. Hendel	A
		Lasiopiophila pilosa Staeger	A
		Parapiophila spp. McAlpine	A
		Prochyliza spp. Walker	A
		Protopiophila latipes (Meigen)	A
		Stearibia nigriceps (Meigen)	A
		Piophilidae spp.	A, I
	Sarcophagidae	*Boettcheria* spp. Parker	A
		Emblemasoma spp. Aldrich	A
		Ravinia spp. Robineau-Desvoidy	A
		Sarcophaga spp. Meigen	A
		Sarcophagidae spp.	A
	Sepsidae	*Meroplius stercorarius* (Robineau-Desvoidy)	A
		Sepsis spp. Fallén	A
		Themira spp. Robineau-Desvoidy	A
	Sphaeroceridae	*Borborillus* spp. Duda	A
		Coproica spp. Ródani	A
		Copromyza equina Fallén	A
		Copromyza stercoraria (Meigen)	A
		Copromyza spp. Duda	A
		Leptocera spp. Olivier	A
		Sphaerocera spp. Latrielle	A
		Sphaeroceridae spp.	A
Coleoptera	Cleridae	*Necrobia rufipes* (DeGeer)	A
	Dermestidae	*Dermestes caninus* Germar	A
		Dermestes maculatus DeGeer	A
		Dermestidae spp.	I
	Histeridae	*Atholus sedecimstriatus* (Say)	A
		Euspilotus assimilis (Paykull)	A
		Esupilotus conformis (LeConte)	A
		Histeridae spp.	A
	Nitidulidae	*Omosita colon* (Linnaeus)	A
	Scarabaeidae	*Onthophagus hecate* (Panzer)	A
		Scarabaeidae spp.	A
	Silphidae	*Necrophila americana* (Linnaeus)	A
		Oiceoptoma noveboracense (Forster)	A
		Thanatophilus lapponicus (Herbst)	A
		Silphidae spp.	I
	Staphylinidae	*Creophilus maxillosus* (Linnaeus)	A
		Omalium spp. Gravenhorst	A
		Staphylinidae sp. 1	A
		Staphylinidae sp. 2	A
		Staphylinidae sp. 3	A
Hymenoptera	Braconidae	Braconidae spp.	A
	Vespidae	Vespidae spp.	A

*A indicates specimens collected as adults; I indicates specimens collected as immatures.

found at the carcasses throughout 2017 and 2018 was collected no more than 7 times. Twice, *Ca. vicina* was collected as larvae and successfully reared to adult from the October 2017 carcass, but no other species was reared from the genus.

Phormia regina was the most common in occurrence as both adults and larvae and was present in both life stages every month of collection, with the exception of the October 2017 carcass during which no larvae were collected. In several trials, *P. regina* larvae were collected the same day that adults were first collected. This species was most often the first to arrive at freshly placed carcasses and the first to have larvae emerge from eggs. *Phormia regina* is the most abundant and widespread blow fly in North America (Whitworth, 2006). Similar research reports *P. regina* to be the most abundant; however, this seems to be influenced by season (Benbow *et al.*, 2013; Tabor *et al.*, 2004).

In the southeast United States where *Lucilia coeruleiviridis* is common (Whitworth, 2006), the species displays high abundance during the hotter temperatures of summer (Benbow *et al.*, 2013; Gruner *et al.*, 2007; Tabor *et al.*, 2004). While *L. coeruleiviridis* did not show the same abundance here in Minnesota, it did occur more often in the hotter months of summer. *Lucilia coeruleiviridis* was not collected until July 2017. Additionally, adults were typically seen on the rest of the 2017 carcasses on the first few days of a trial, which aligns with similar studies that concluded the species is most abundant in the fresh stage of decomposition (Cruise *et al.*, 2018; Gruner *et al.*, 2007; Tabor *et al.*, 2004). *Lucilia coeruleiviridis* was often present in the earliest days of a trial in other faunal surveys (Benbow *et al.*, 2013).

Lucilia illustris occurred infrequently as an adult and only twice as larvae. *Lucilia illustris* larvae were collected from the August and September 2017 carcasses while both were in fresh decomposition. Adults were only collected six other times, with three of those occurrences from the August 2017 carcass. *Lucilia illustris* was found only once in 2018 on the July carcass. The infrequent collection of *L. illustris* is interesting considering that it is a common and geographically widespread species (Whitworth, 2006), and Table 2.3 shows it present in six other studies.

Like *Phormia regina*, *Lucilia sericata* was found often throughout both years of collection as both adults and larvae; adults were collected from every carcass and *L. sericata* larvae were also collected from every carcass. This species' larvae were also collected from inside the skull of the October 2017 carcass as late as 2 December. However, *L. sericata* larvae were not collected from the same carcass in the following thaw of spring 2018 despite the presence of adults. The possibility that the larvae overwintered in the carcass and dispersed prior to spring collection began opens the door for further research. This species is the most common within its genus and is found throughout the United States (Whitworth, 2006).

Lucilia silvarum was collected a total of four times, with the first collection on the May 2017 carcass in fresh stage of decomposition. *Lucilia silvarum* is a common species found throughout North America; however, it is a known parasite of toads, thus, there is limited literature on whether the species should be considered forensically important (Fremdt *et al.*, 2012). The presence of adult specimens of *L. silvarum* is possibly significant, as it is not recognized as a forensically important colonizer of carrion in the United States (Adair & Kondratieff, 2006; Fremdt *et al.*, 2012). In a rare finding of the primary colonization of *L. silvarum* on a body found in high elevation Colorado, Adair and Kondratieff (2006) discuss the possibility that colonization occurred due to lack of competition. All collections of this species occurred during the fresh stage when other adult species and larvae were present. It is possible that *L. silvarum* was present with the purpose of colonizing the carcass but was unsuccessful due to competition. *Lucilia silvarum* is not found in similar research (Benbow *et al.*, 2013; Cruise *et al.*, 2018; Gruner *et al.*, 2007; Tabor *et al.*, 2004). Nevertheless, the collections on the carcasses of this study could suggest that further investigation is needed to determine the forensic relevance of this species.

Table 2.3 shows Calliphoridae by region and includes the findings of the present study. Geographic locations include British Columbia, Canada and Hawaii, Colorado, Minnesota, Michigan, Ohio, Louisiana, Mississippi, Virginia, North Carolina, and Florida (Anderson & Vanlaerhoven, 1996; Benbow *et al.*, 2013; Cruise *et al.*, 2018; DeJong & Chadwick, 1999; Early & Goff, 1986; Goddard *et al.*, 2012; Gruner *et al.*, 2007; Pastula & Merritt, 2013; Tabor *et al.*, 2004; Watson & Carlton, 2003). The table is organized to show presence and absence of calliphorid species from the west to the east of North America. The total column along the right of the table lists the number of states or provinces the corresponding species has occurred and the total taxa row along the bottom of the table shows the number of species recorded for that state or province's research.

As expected, Minnesota shares common, widespread species such as *Phormia regina*, *Cochliomyia macellaria*, *Lucilia illustris* and *L. sericata* with other regions across the continent (Table 2.3). However, Table 2.3 highlights the key role that geographic range plays in the presence of species like *Lucilia coeruleiviridis* and *Protophormia terraenovae*, whose geographic range overlaps, but ends with Minnesota. It is important to notice that Minnesota is the only study that records the combination of these two species, showing a unique distributional overlap. Taking a closer look at the studies located nearest to Minnesota, we see some overlap of species with Colorado, such as *Calliphora terraenovae*, *Ca. vicina*, *Ca. vomitoria*, *Cochliomyia macellaria*, *Phormia regina*, *Lucilia illustris* and *L. sericata*—all of which are common and widespread species—and we see a total overlap with all species recorded in Michigan. However, I recorded species here in Minnesota, *Calliphora latifrons* and *Lucilia silvarum*, that you would expect to find in Colorado and Michigan but do not. These findings support the importance of further insect succession research in an effort to compile complete geographic record of these carrion-visiting Calliphoridae.

Table 2.3 Calliphoridae collected on decomposing carcasses in North America by state or province including new results from 11 decomposing pig carcasses. University of Minnesota St. Paul dairy, spring 2017 to fall 2018.

		West			Central			South					
Subfamily	Species	HI	BC	CO	**MN**	MI	OH	LA	MS	VA	NC	FL	# Studies
Calliphorinae	*Calliphora alaskensis*	-	-	+	-	-	-	-	-	-	-	-	1
	Calliphora coloradensis	-	-	+	-	-	-	-	-	-	-	-	1
	Calliphora latifrons	-	+	-	**+**	-	-	-	-	-	-	-	2
	Calliphora lilaea	-	-	+	-	-	-	-	-	-	-	-	1
	Calliphora livida	-	-	+	-	-	-	-	-	-	-	+	2
	Calliphora terraenovae	-	-	+	**+**	-	-	-	-	-	-	-	2
	Calliphora vicina	-	+	+	**++**	-	-	-	-	+	-	+	5
	Calliphora vomitoria	-	-	+	**+**	-	-	-	-	+	-	-	3
	Cynomya cadaverina	-	+	+	**+**	-	-	-	-	-	-	-	3
Chrysomyinae	*Chrysomya megacephala*	+	-	-	-	-	-	-	-	-	-	+	2
	Chrysomya rufifacies	+	-	-	-	-	-	+	-	-	-	+	3
	Cochliomyia macellaria	-	-	+	**++**	-	+	+	+	-	+	+	7
	Phormia regina	-	+	+	**++**	+	+	+	+	+	+	+	10
	Protophormia terraenovae	-	+	+	**+**	-	-	-	-	-	-	-	3
Luciliinae	*Lucilia coeruleiviridis*	-	-	-	**++**	+	+	+	+	+	+	+	8
	Lucilia cuprina	+	-	-	-	-	-	+	+	-	+	-	4
	Lucilia illustris	-	+	+	**++**	+	+	-	-	+	+	-	7
	Lucilia sericata	-	+	+	**++**	-	+	+	-	+	+	-	7
	Lucilia silvarum	-	-	-	**+**	-	-	-	-	-	-	-	1
	Total taxa:	3	7	13	**12**	3	5	6	4	6	6	7	

++ indicates collection as both adult and larva

2.3.2 Non-Calliphoridae Diptera

Non-calliphorid Diptera included five forensically important Diptera families (Table 2.2), in decreasing order of taxon abundance: Sphaeroceridae, Piophilidae, Muscidae, Sarcophagidae, and Sepsidae. These five families are recorded in similar initial succession research, recorded at family-level identification (Benbow *et al.*, 2013; Cruise *et al.*, 2018; Tabor *et al.*, 2004; Watson & Carlton, 2003).

The most diverse non-calliphorid Diptera taxa represented was Sphaeroceridae with seven taxa identified to either genus or species. *Coproica*, *Copromyza*, *Leptocera*, *Sphaerocera*, and *Borborillus* were recorded here in Minnesota. Similar faunal studies in Hawaii (Goff *et al.*, 1986), Vancouver (Anderson & Vanlaerhoven, 1996), Colorado (DeJong & Chadwick, 1999), Michigan (Pastula & Merritt, 2013) and Louisiana (Watson & Carlton, 2003) also recorded *Leptocera*, although the genus has over 50 species.

Piophilidae displayed the second greatest species diversity with 6 taxa identified to genus or species. *Prochyliza*, and *Stearibia nigriceps* (Meigen) are recorded as present on carcasses in this research as well as in similar studies in Mississippi, Virginia and Louisiana (Goddard *et al.*, 2012; Tabor *et al.*, 2004; Watson & Carlton, 2003).

Five Muscidae taxa were identified to genus only. *Musca* species are recorded in Minnesota and in Hawaii (Goff *et al.*, 1986), Virginia (Tabor *et al.*, 2004), and North Carolina (Cruise *et al.*, 2018). *Hydrotaea* are recorded in Minnesota as well as various succession studies across the continent (Anderson & Vanlaerhoven, 1996; DeJong & Chadwick, 1999; Pastula & Merritt, 2013; Tabor *et al.*, 2004; Watson & Carlton, 2003).

Sarcophagidae were present with four identified taxa. Sarcophagidae at family level have been recorded (Benbow *et al.*, 2013; Cruise *et al.*, 2018) at carcasses, and *Boettcheria*, *Ravinia*, and *Sarcophaga* are recorded in this study as well as faunal studies research in Virginia (Tabor *et al.*, 2004).

Three taxa in Sepsidae were identified. Similar studies reported the same taxa as did this research: *Sepsis* (Goff *et al.*, 1986; Tabor *et al.*, 2004; Watson & Carlton, 2003) and *Meroplius stercorarius* (Robineau-Desvoidy) (Watson & Carlton, 2003).

2.3.3 Coleoptera

Coleoptera found in this study are compared with that of similar initial studies research. Each taxon found within the order Coleoptera is referenced in Table 2.2 with identification to genus and species level.

Staphylinidae were represented with the most diversity with five morphotypes identified. I recorded *Creophilus maxillosus* (L.) in Minnesota, as well as an *Omalium* species and three additional morphotypes. *Creophilus maxillosus* and family-level identification of Staphylinidae was recorded in similar studies (Anderson & Vanlaerhoven, 1996; Benbow *et al.*, 2013; Cruise *et al.*, 2018; DeJong & Chadwick, 1999; Goddard *et al.*, 2012; Pastula & Merritt, 2013; Tabor *et al.*, 2004; Watson & Carlton, 2003).

Silphidae and Histeridae are each represented by three taxa. Silphid adults of *Necrophila 32mericana* (L.), *Oiceoptoma noveboracence* (Forster)and *Thanatophilus lapponicus* (Herbst) were recorded in Minnesota. *Necrophila 32mericana* are recorded in several studies across the continent (Cruise *et al.*, 2018; Pastula & Merritt, 2013; Tabor *et al.*, 2004; Watson & Carlton, 2003), while *Oiceoptoma noveboracence* is only recorded in faunal studies in Ohio and Virginia (Benbow *et al.*, 2013; Tabor *et al.*, 2004) Additionally, *Thanatophilus lapponicus* is only recorded in Vancouver and Colorado (Anderson & Vanlaerhoven, 1996; DeJong & Chadwick, 1999). This finding is not too surprising, as the geographic range of *T. lapponicus* includes most of Canada and the northern United States (Ratcliffe, 1996).

Histeridae are recorded at family level (Benbow *et al.*, 2013; Cruise *et al.*, 2018), and the species *Euspilotus assimilis* (Paykull) was found on carcasses in similar faunal surveys (Tabor *et al.*, 2004; Watson & Carlton, 2003). However, *Euspilotus conformis* (LeConte) is scarce in similar succession literature and its recorded range falls a few states short of Minnesota to the east (Dillon, 1961); however, there is physical record of specimens collected in Minnesota (University of Minnesota Insect Collection, personal observation).

Dermestidae are represented by two taxa identified to species. Similar research recorded dermestids at family level (Cruise *et al.*, 2018), the genus *Dermestes* (Tabor *et al.*, 2004), and species *Dermestes caninus* Germar (Watson & Carlton, 2003). *Dermestes maculatus* DeGeer was recorded in succession research in Hawaii (Early & Goff, 1986; Goff *et al.*, 1986), but the species is not reported in other faunal studies. The species is common and widespread, making this lack of occurrence in research puzzling. It may be that a portion of specimens that literature identifies only as *Dermestes sp.* Were actually specimens of D. *maculatus*.

Cleridae, Nitidulidae, and Scarabaeidae are each represented by one taxon identified to species. Cleridae are referenced in similar literature as *Necrobia rufipes* (DeGeer) (Anderson & Vanlaerhoven, 1996; Goddard *et al.*, 2012; Goff *et al.*, 1986; Pastula & Merritt, 2013; Tabor *et al.*, 2004; Watson & Carlton, 2003). Scarabaeidae are referenced to family-level (Benbow *et al.*, 2013) and species *Onthophagus hecate* (Panzer) in succession studies literature

(Cruise *et al.*, 2018; Watson & Carlton, 2003). *Omosita colon* is recorded in initial succession studies (Benbow *et al.*, 2013; Watson & Carlton, 2003).

2.4 Conclusion

With the results of this chapter, I have produced a reference checklist of forensically important insect fauna that are specific to this geographic region. This checklist adds to the valuable insect succession findings that exists in North America and is the first of its kind in Minnesota. This checklist is like that of other regional faunal studies when comparing species that are widespread; however, it highlights the differences that a geographic range of species produces. The record of *Lucilia silvarum* at my carcasses is unique thus far and could be further investigated as a forensically important species of Calliphoridae. This checklist of forensically important insects that visit decomposing bodies is unique to Minnesota and is the first for the surrounding region and can be considered a useful comprehensive tool for the practical applications of local law enforcement agencies and FE's investigating unnatural death in the upper Midwest. Furthermore, the introduction of this checklist brings focus to the need for additional faunal succession studies throughout North America, and perhaps even more necessary in the upper Midwest, as the region lacks faunal study data, and may hold unique patterns of range overlap of species. Future faunal studies in Minnesota would be beneficial in examining whether the region holds a unique grouping of calliphorid species.

The following chapter will elaborate on this checklist by considering the effects of decomposition and seasonality in Minnesota.

Chapter III: Decomposition and Seasonal Distribution

3.1 Introduction

Insect evidence collected at a scene of death can be useful in determining a timeline of insect colonization of a body (Hall, 2001). An approximate timeline after death can be put together by looking at the temperature-dependent development of Calliphoridae throughout their life cycle; however, the succession of insects that occurs throughout the predictable stages of decomposition can also answer questions concerning time of death. Insect succession research in various parts of North America has shown the taxa that are present through the stages of decomposition, highlighting geographic differences and seasonal differences of specific geographic locations, but none has been published from Minnesota. Chapter II investigated the insect taxa that occur on decomposing pig carcasses in Minnesota and presented a reference list of all insect taxa that occurred on carcasses regardless of stage of decomposition or season. To compare what is found in Minnesota to that of other research, I will analyze the sequence of insect taxa by stage of decomposition and seasonality.

3.2 Materials and Methods

Freshly killed pigs were placed on site monthly during non-winter months from May 2017 through September 2018 (Table 2.1), and I collected forensically important insect taxa of all life stages daily by aerial netting, manual collection, and pitfall traps. A portion of calliphorid larvae was preserved, and another portion was reared to identify them as adults. All other insect taxa were preserved. Weather observations and the stage of carcass decomposition, as defined in Chapter I (Figure 3.1), were recorded daily.

Figure 3.1 July 2018 pig carcass in the first four stages of decomposition. University of Minnesota St. Paul campus dairy. (a) fresh stage of decomposition; (b) bloat stage; (c) active decay stage; (d) advanced decay stage

In 2017, sampling continued through all stages of decomposition. In 2018, I focused more on Calliphoridae, and as a result, carcasses were removed during advanced decay stage when calliphorid activity decreased. Therefore, presence data for insects are only available through active decay stage in 2018.

For simplicity, I define meteorological seasons as spring (1 March through 31 May), summer (1 June through 31 August), fall (1 September through 30 November), and winter (1 December through 28 February). It is important to note that the number of collecting days in each season differ, simply because insects are not present for portions of fall, winter, and spring due to decreased temperatures. Spring had 62 days in which collection was possible, summer had 184 collection days, and fall had 80. Collection ceased once freezing weather halted insect activity and physical decomposition. I left the October 2017 carcass at the site over winter to observe decomposition and insect succession as snow cover receded in spring, 2018. Complete temperature data can be referenced in Appendix A.

3.3 Results & Discussion

3.3.1 Decomposition

Table 3.1 shows in detail how many days each carcass was in each stage of decomposition from spring 2017 to fall 2018. Data was not recorded for the entirety of advanced decay for all the carcasses, so the values for this stage are not included in the totals. These values are intended to convey a potential relative abundance for taxa present through the stages of decomposition. Though collection was thorough, and effort was made to ensure all taxa present were collected, it was impossible to collect every single insect present.

Summer carcasses progressed the fastest through the stages of decomposition, overall. The shortest amount of time to reach the beginning of advanced decay was 7 days; this occurred four times, with the June and July 2017 carcasses, and the July and August 2018 carcasses (Table 3.1). The October 2017 carcass took the longest time to reach the beginning of advanced decay because this carcass stayed out for the duration of winter. Discounting the overwintered carcass, the longest amount of time to reach the beginning of advanced decay was the May 2017 carcass with a total time of 22 days spent in the first three stages of decomposition. Due to an increased range of hourly temperatures in the month of May, which is characteristic in the state of Minnesota (personal observation), this longer duration of decomposition is not surprising (Appendix A). Other initial succession research describes longer fresh stages of decomposition due to decreased temperatures (Cruise *et al.*, 2018). Payne (1965) showed that insect activity on a carcass influences its rate of decomposition. This, along with the temperature-dependent development of initial colonizers, explains the longer duration of decomposition for spring carcasses.

The October 2017 carcass remained in fresh stage until spring 2018 when snow cover receded, and temperatures increased (Appendix A). However, a bloat stage was not directly observed in spring, and if it did occur it lasted less than a day. Additionally, the active decay stage of this carcass lasted considerably longer than the May 2018 carcass set out at the same time when spring sampling resumed for the October 2017 carcass.

Table 3.1 Number of days each carcass spent in stage of decomposition. University of Minnesota St. Paul campus dairy, spring 2017 to fall 2018.

	Stage of Decomposition			
	Fresh	Bloat	Active Decay	Total
May 2017	5	4	13	22
June 2017	1	3	3	7
July 2017	2	2	3	7
August 2017	4	2	4	10
September 2017	2	4	3	9
October 2017	195	0	22	217
May 2018	5	4	6	15
June 2018	1	5	3	9
July 2018	1	2	4	7
August 2018	4	1	2	7
September 2018	2	1	7	10

Table 3.2 shows the percentage of carcasses on which each insect taxon was collected, by stage of decomposition and is organized by order and shows the ecological role of each family. The percentage of presence on a carcass is shown for each taxon and stage of decomposition. For example, looking at *Lucilia sericata*, we see that this taxon was present on 91%, or 10 out of the 11 carcasses for every stage of decomposition with the exception of dry remains stage.

Special attention is brought to Calliphoridae which were identified to species, while other taxa are grouped to family in this objective. This focus on Calliphoridae follows that of other insect succession research (Benbow *et al.*, 2013; Gruner *et al.*, 2007), as Calliphoridae are typically the initial colonizers of a body. All insect taxa collected were identified to either genus or species; refer to Table 2.2 for a complete taxon reference list.

Table 3.2 groups all species of Calliphoridae, all other families of Diptera, all families of Coleoptera, and all families of Hymenoptera. The functional groups outlined in Chapter II are included for each taxon listed. Additionally, taxa are listed within their sections by order of decreasing occurrence percentages to highlight the succession through the stages of decomposition in relation to functional group.

Calliphoridae have higher percentages in the earlier stages of decomposition and percentages of Coleopteran families increase in the later stages of decomposition. This expectation follows that of similar research in which ecological role relates to stage of decomposition.

Based on results from earlier studies (Benbow *et al.*, 2013; Cruise *et al.*, 2018; Tabor *et al.*, 2004; Watson & Carlton, 2003), I predicted that the initial colonizers of a carcass would dominate during: fresh, bloat, and active decay stages. As expected, initial colonizers, or necrophages, were most abundant in the first three stages of decomposition; whereas the predators that consume these prey items are most abundant in the mid to late stages of decomposition. The percentage of collection events throughout Table 3.2 highlights the succession predicted based on ecological role. Again, these findings are consistent with similar succession research examining insect fauna of forensic importance through stage of decomposition, which shows calliphorid abundance in the earlier stages of decomposition (Benbow *et al.*, 2013; Cruise *et al.*, 2018; Payne, 1965; Tabor *et al.*, 2004; Watson & Carlton, 2003).

Within Calliphoridae, *Phormia regina* and *Lucilia sericata* was collected most frequently, with the highest collections from fresh to advanced decay stages of decomposition with numbers decreasing in the dry remains stage of decomposition. These numbers were expected in the first three stages of decomposition. However, presence of calliphorid adults in later stages of decomposition was surprising. The presence in advanced decay may be due to collection of larvae that had yet to disperse from the carcass and were sparse in number, or presence in later stages were simply visiting blow flies to the area. Dry remains collections of calliphorid adults were most likely due to the presence of neighboring carcasses and the aerial mobility of Diptera. There were no observations of adult calliphorids ovipositing during advanced decay or dry remains.

Lucilia sericata was found on all but one carcass through advanced decay. *Phormia regina* presence was high throughout these stages of decomposition and was the only calliphorid species found on every carcass during active decay.

Most non-calliphorids were consistent by presence throughout all stages of decomposition. Despite similar research observing muscid larvae (Watson & Carlton, 2003), I saw no evidence of colonization by any other Diptera families besides Calliphoridae on any of the 11 carcasses.

Sarcophagid adults (Table 2.2) were rarely collected, with only a few collection events in advanced decay and dry remains (Table 3.2). These few records and their absence in early stages of decomposition are surprising considering that Sarcophagidae are known to be early visitors to decomposing carcasses (Cruise *et al.*, 2018; Tabor *et al.*, 2004; Watson & Carlton, 2003). Competition of resources possibly explains the absence of sarcophagid adults and larvae in the early stages of decomposition (Denno & Cothran, 1976); though, incompatible rearing methods for sarcophagid larvae could also be further examined for the absence of sarcophagid larvae and adults.

I expected Coleoptera to occur in greater abundance in the later stages of decomposition, as shown in similar studies (Benbow *et al.*, 2013; Cruise *et al.*, 2018; Tabor *et al.*, 2004; Watson & Carlton, 2003), as most are predators. The greatest number of collection events were in the later stages of decomposition, and least in early stages (Table 3.2). Staphylinidae and Histeridae often occurred sporadically as soon as calliphorid larvae were present, with collection increasing in active decay when calliphorid larvae were most abundant.

Omnivorous coleopteran adults, such as Silphidae and Scarabaeidae, eat calliphorid larvae, but will exploit the carcass as scavengers in their immature stages (Cruise *et al.*, 2018). Table 3.2 shows consistent presence on the carcasses throughout the stages of decomposition with a slight increase in collection in the later stages of decomposition, supporting their expected ecological roles on the carcass.

The necrophagous Dermestidae arrived in the later stages of decomposition of advanced decay and dry remains, much like similar succession research that reported the same (Cruise *et al.*, 2018). It should be noted that the 2018 collections were halted at the advanced decay stage of decomposition; this most likely affected the number of collections reported of Dermestidae and possibly other insect taxa in the dry remains column of Table 3.2.

Where the results of the percentage of occurrences through the stages of decomposition differ from existing similar research lies within the details of geographic range of species rather than the larger idea of insect succession. For example, research in Ohio lists *Lucilia coeruleiviridis* to be most abundant calliphorid adults in the fresh stages of decomposition (Benbow *et al.*, 2013), while here in Minnesota, the percentages for *Lucilia sericata* and *Phormia regina* are dominant in fresh stage (Table 3.2). *Lucilia coeruleiviridis* is found here, but as Minnesota nears the edge of the species' geographic range (Whitworth, 2006), is not as abundant as *P. regina* and *L. sericata*.

Table 3.2 Percentage of pig carcasses (n = 11) on which insect taxa occurred, by stage of decomposition. University of Minnesota St. Paul campus dairy, spring 2017 to fall 2018.

Order	Family	Taxon	Functional Group	Fresh	Bloat	Active	Adv	Dry
						%		
Diptera	Calliphoridae	*Lucilia sericata*	Necrophage	91	91	91	91	55
Diptera	Calliphoridae	*Phormia regina*	Necrophage	82	91	100	82	55
Diptera	Calliphoridae	*Cochliomyia macellaria*	Necrophage	18	55	64	27	9
Diptera	Calliphoridae	*Lucilia coeruleiviridis*	Necrophage	55	27			18
Diptera	Calliphoridae	*Lucilia illustris*	Necrophage	27	9	9		9
Diptera	Calliphoridae	*Calliphora vomitoria*	Necrophage	18	18			
Diptera	Calliphoridae	*Lucilia silvarum*	Necrophage	18		9		9
Diptera	Calliphoridae	*Calliphora terraenovae*	Necrophage			9		18
Diptera	Calliphoridae	*Calliphora latifrons*	Necrophage		9			9
Diptera	Calliphoridae	Calliphoridae spp.	Necrophage				9	
Diptera	Calliphoridae	*Calliphora vicina*	Necrophage	9				
Diptera	Calliphoridae	*Cynomya cadaverina*	Necrophage	9				
Diptera	Calliphoridae	*Protophormia terraenovae*	Necrophage		9			
Diptera	Muscidae	Muscidae spp.	Necrophage	55	73	9	73	46
Diptera	Piophilidae	Piophilidae spp.	Necrophage	18	27	46	55	36
Diptera	Sepsidae	Sepsidae spp.	Necrophage	18	18	36	18	27
Diptera	Sphaeroceridae	Sphaeroceridae spp.	Necrophage	18	18	27	27	27
Diptera	Sarcophagidae	Sarcophagidae spp.	Necrophage				9	27
Coleoptera	Nitidulidae	Nitidulidae sp.	Necrophage		18	9	36	46
Coleoptera	Dermestidae	Dermestidae spp.	Necrophage				27	36
Coleoptera	Silphidae	Silphidae spp.	Omnivore	36	36	36	82	55
Coleoptera	Scarabaeidae	Scarabaeidae spp.	Omnivore	9	18	9	18	27
Coleoptera	Staphylinidae	Staphylinidae spp.	Predator	18	64	73	73	55
Coleoptera	Histeridae	Histeridae spp.	Predator	9	36	55	46	36
Coleoptera	Cleridae	Cleridae sp.	Predator			46	82	55
Hymenoptera	Vespidae	Vespidae spp.	Omnivore	27	27	9		
Hymenoptera	Braconidae	Braconidae spp.	Parasitoid		18	27		

n = 6 in 2017, 5 in 2018
*data for 2018 "Dry Remains" are not complete

3.3.2 Seasonal Distribution

The season these taxa visited the carcasses is represented in Table 3.3 in which presence is tallied per taxa per season. I define presence as a singular day of any season of which a taxon was collected. To elaborate, these values do not represent the number of individuals collected in total but is a record of the number of days within a season that the taxa was collected. This research does not provide a quantitative value, but rather a qualitative relative abundance to show how many days in a season that a taxon was collected for the entirety of 2017 and 2018.

The total column on the right of the table is the sum of the days each individual taxon was collected per season for the entirety of 2017 and 2018. The Total taxa row at the bottom of the table is the number of taxa collected per season. These values are intended to convey a relative abundance for taxa present through the seasons and do not reflect quantitative findings. As stated in section 3.3.1, though collection was thorough, and an effort was put forth to ensuring all taxa present were collected, it was impossible to collect every single insect present.

Seven calliphorid species were collected in both spring and summer, and 11 calliphorid species were collected in fall. Table 3.3 includes both larval and adult Calliphoridae together as one value. For a more in-depth focus on calliphorid life stage in relation to season, see Tables 3.4 and 3.5.

Phormia regina and *Lucilia sericata* adults were consistently most abundant through the three warm seasons. Other *Lucilia* adults were collected sporadically through summer and fall. *Cochliomyia macellaria* adults were abundant in summer. *Calliphora* spp. were most abundant in spring and fall, though overall collection was rare in these seasons, and there were no reared *Calliphora* species. An adult *Protophormia terraenovae,* a species considered to be common on carrion in the northern U.S. (Whitworth, 2006), was collected only once in the spring.

During an unseasonable winter warming of temperatures in December 2017, larvae were expected to be active within the carcass due to the increase in temperatures and the observation of adult fly activity in the area. With temperatures at 10°C, I confirmed this idea by opening the skull and the thorax of the carcass and collecting live calliphorid larvae. These larvae were reared to adult and identified to *Lucilia sericata*.

Taxa from families other than Calliphoridae generally showed an increase in collection in the summer, with the exception of Sarcophagidae. This trend was apparent among families of Coleoptera collected.

The results of this objective show a parallel similarity to other research that has examined seasonal distribution for geographically overlapping species of Calliphoridae. *Phormia regina* shows the highest number of days of collection in both spring and summer (Table 3.3), matching abundance patterns in seasonal distribution research in Virginia (Tabor *et al.*, 2004). This abundance decreased in fall but was generally higher relative to most calliphorid species collected. However, this decrease in days of collection in the fall contradicts *P. regina's* life history as a cold weather species (Byrd & Castner, 2001).

Lucilia sericata occurred less frequently than *P. regina* in spring collections but was more frequent than all other remaining Calliphoridae caught in this season. *Lucilia sericata* increased greatly in summer and surpassed the abundance of *P. regina* in fall. A study in Florida reports the highest abundance of calliphorid species throughout summer collection to be *Lucilia coeruleiviridis*, the *Lucilia* species most common to that geographical range (Gruner *et al.*, 2007). While *L. coeruleiviridis* was found in Minnesota (Table 2.1), it was not as common as *L. sericata* (Table 3.3).

Table 3.3 Number of collection events of forensically important insect taxa on 11 decomposing pig carcasses, grouped by season at time of collection. University of Minnesota St. Paul campus dairy, Spring 2017 to Fall 2018.

Order	Family	Species/Family	Spring	Summer	Fall	Winter	Total
			n = 62	*n* = 184	*n* = 80	*n* = 1	
Diptera	Calliphoridae	*Phormia regina*	38	54	12		104
		*P. regina**	47	54	10		111
		Lucilia sericata	25	58	25	1	109
		*L. sericata**	9	28	20		57
		Cochliomyia macellaria		12	5		17
		*Co. macellaria**		16	9		25
		Lucilia coeruleiviridis		6	9		15
		*L. coeruleiviridis**		2	1		3
		Lucilia illustris		2	4		6
		*L. illustris**		1	1		2
		Calliphora vomitoria	1	1	5		7
		Calliphora terraenovae	3		2		5
		Calliphora vicina			2		2
		*C. vicina**			2		2
		Lucilia silvarum	1	2	1		4
		Cynomya cadaverina	1		2		3
		Calliphora latifrons			2		2
		Protophormia terraenovae	1				1
		Subtotal of taxa per season:	7	7	11	1	
Diptera	Muscidae	Muscidae spp.	29	70	26		125
Diptera	Piophilidae	Piophilidae spp.	5	25	14		44
Diptera	Sepsidae	Sepsidae spp.	15	13			28
Diptera	Sphaeroceridae	Sphaeroceridae spp.	7	16	2		25
Diptera	Sarcophagidae	Sarcophagidae spp.		5	1		6
		Subtotal of taxa per season:	4	5	4		
Coleoptera	Silphidae	Silphidae spp.	5	111	9		125
Coleoptera	Silphidae	Silphidae spp.*		79	5		84
Coleoptera	Staphylinidae	Staphylinidae spp.	27	108	34		169
Coleoptera	Nitidulidae	Nitidulidae sp.	5	70	17		92
Coleoptera	Dermestidae	Dermestidae spp.		43	2		45
Coleoptera	Dermestidae	Dermestidae spp.*		41			41
Coleoptera	Cleridae	Cleridae sp.	4	35	22		61
Coleoptera	Histeridae	Histeridae spp.	5	22	6		33
Coleoptera	Scarabaeidae	Scarabaeidae spp.	7	9	3		19
		Subtotal of taxa per season:	6	7	6		
Hymenoptera	Vespidae	Vespidae spp.	1	2	7		10
Hymenoptera	Braconidae	Braconidae spp.		1	1		2
		Subtotal of taxa per season:	1	2	2		
		Total taxa per season:	18	21	23	1	

*indicates larval collection

Protophormia terraenovae is cited as the most cold-tolerant of all calliphorid species and is reported to be very common during cool weather in the northern U.S. (Byrd & Castner, 2001; Grassberger & Reiter, 2002). Yet, the species was collected only once in this study during the spring. The single collection occurred on Day 6 of carcass placement with ambient temperature at 24°C.

Also known for their cold-hardiness are *Calliphora spp*. (Ames & Turner, 2003; Donovan *et al.*, 2006; Faucherre *et al.*, 1999). Unsurprisingly, *Calliphora* species were collected in the cooler seasons of spring and fall; however, the abundance was lower than expected. Table 3.3 shows these trends, apart from one collection of *Calliphora vomitoria* in summer. However, summer collection of *C. vomitoria* was on a cooler, overcast day with an ambient temperature of 20°C at the time of collection.

Table 3.4 focuses on calliphorid adults collected from the 11 pig carcasses from spring 2017 to fall 2018. The presence or absence is noted by a “plus” or “minus”. This table suggests the chance of collecting a given species may rely on season. Eight calliphorid species were collected in spring, and 7 calliphorid species were recorded in summer. Interestingly, all species of existing record from this study were recorded in fall with the exception of *Protophormia terraenovae*.

Table 3.4 Occurrence of adult Calliphoridae collected from 11 decomposing pig carcasses. University of Minnesota, St. Paul campus dairy, Spring 2017 to Fall 2018.

Species	Spring	Summer	Fall
Phormia regina	+	+	+
Lucilia sericata	+	+	+
Cochliomyia macellaria	+	+	+
Lucilia silvarum	+	+	+
Calliphora vomitoria	+	+	+
Calliphora terraenovae	+	-	+
Cynomya cadaverina	+	-	+
Protophormia terraenovae	+	-	-
Lucilia coeruleiviridis	-	+	+
Lucilia illustris	-	+	+
Calliphora latifrons	-	-	+
Calliphora vicina	-	-	+

Table 3.5 focuses on reared Calliphoridae and denotes their presence or absence throughout the seasons from spring 2017 to fall 2017 with a “plus” or “minus”. Species are listed by abundance of reared specimens.

Few calliphorid species were reared in large numbers from collected larvae. In order of relative abundance shown in Table 3.5: *Phormia regina, Lucilia sericata, Cochliomyia macellaria, L. coeruleiviridis, L. illustris*, and *Calliphora vicina*. *Phormia regina* was encountered in all seasons except winter. *Lucilia sericata* was encountered in all seasons. *Cochliomyia macellaria, L. coeruleiviridis,* and *L. illustris* were found in summer and fall. *Calliphora vicina* was collected only in the fall from the October 2017 carcass during fresh stage of decomposition. The cooler temperatures at both collection events, 14.4°C and 20.5°C respectively, reflect the cold-hardy nature of the *Calliphora* genus (Appendix A).

Phormia regina was the most abundant larval encounter, with more than 100 specimens successfully reared to adult. *Lucilia sericata* was next in larval collection abundance with slightly over 50 specimens successfully reared to adult, and *Cochliomyia macellaria* followed with 21 reared specimens (Table 3.5). However, the remaining species collected as larvae and reared to adult are much lower in the number of successfully reared specimens, with *Lucilia coeruleiviridis* reared successfully three times, and both *Calliphora vicina* and *Lucilia illustris* reared successfully just two times. It is surprising that any species would be reared in such low abundance. However, it is likely that some species, such as *P. regina* and *L. sericata*, reared particularly well in lab conditions, whereas other species may not have been as successful.

Table 3.5 Occurrence of reared Calliphoridae collected from 11 decomposing pig carcasses; University of Minnesota St. Paul campus dairy, Spring 2017 to Fall 2018.

Species	Spring	Summer	Fall	Winter
Phormia regina	+	+	+	-
Lucilia sericata	+	+	+	+
Cochliomyia macellaria	-	+	+	-
Lucilia coeruleiviridis	-	+	+	-
Lucilia illustris	-	+	+	-
Calliphora vicina	-	-	+	-

Diptera other than Calliphoridae were steadily collected through all seasons, apart from Sarcophagidae. Sarcophagid specimens were collected very rarely across all seasons of the two years the study was conducted. Muscidae were most abundant in collections, most of which were *Hydrotaea* spp. (Table 2.2). These flies are common on carrion, as a faunal study in Louisiana (Watson & Carlton, 2003).

Encounters with Coleoptera increased in summer. Silphidae and Staphylinidae were most frequent. No Dermestidae were collected in the spring;

however, encounters increased greatly in summer, then decreased to only two encounters in fall. *Dermestes caninus* and *Dermestes maculatus*; both species common to carrion and occur in the Midwest, were the only two dermestid species collected (Table 2.2). Dermestidae are known to become active in summer (Byrd & Castner, 2001).

The findings of these objectives were expected to align with similar succession research from regions of North America. Acknowledging some variation of temperature range for each season, I did not believe these differences in temperatures would be significant enough to affect the calliphorid species that are most seasonally abundant; however, these findings add valuable data to our comprehension of insect succession. The most noteworthy difference in seasonal abundance of calliphorid adults is the result of geographic range, highlighted in Chapter II, which shows a unique combination of calliphorid species recorded here in Minnesota. The described successional patterns of recorded insect taxa in relation to the stages of decomposition and the seasons of Minnesota strengthens existing similar regional insect succession research.

3.4 Conclusion

The data collected from this project adds to the valuable insect succession findings that exists in North America. This chapter elaborates on the forensically important taxa list regarding both stage of decomposition of which these taxa appear and the season in which they appear. Overall, the findings of this research consistently align with initial faunal surveys that examine stage of decomposition and season (Benbow *et al.*, 2013; Cruise *et al.*, 2018; Gruner *et al.*, 2007; Tabor *et al.*, 2004; Watson & Carlton, 2003), and the hypothesized expectations of which taxa are present through stages of decomposition and with the seasons of Minnesota. However, this chapter brings to light some surprising results unique to Minnesota that invite further investigation. One example concerns the presence of *Lucilia sericata* larvae found within a carcass in early December 2017, and the near absence of adult Sarcophagidae collection for the entirety of the study. Further study could examine whether calliphorid larvae are able to overwinter within a decomposing carcass in the frigid winter temperatures of Minnesota, and further examination could shed light on why Sarcophagidae were nearly absent throughout this study.

This research is the first project to examine the succession of forensically important insects that occur on decomposing carcasses in Minnesota. Additionally, this is the first project to span every month of the year with permissive temperatures. While similar research has examined the seasonal effects of decomposition (Benbow *et al.*, 2013), this project is comprised of monthly trials that allowed a closer look at how the decomposition aligned with the Minnesota seasons.

In conjunction with Chapter II, this chapter and its sections are compiled to assist in legal investigations concerning insects of forensic importance. The results of this thesis can assist in answering questions surrounding unnatural death. Chapter II provides a faunal checklist that can help in determining whether a body has been moved postmortem. Chapter III provides information that can assist in determining *when* did death occur. Local law enforcement agencies and consulting FE’s will have this thesis at their disposal to use as a tool of reference for the geographically specific fauna that visit cadavers here in Minnesota.

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APPENDIX A

Daily average temperatures measured by a HOBO data logger with probes measuring ambient air temperature, soil temperature, and internal carcasses temperatures. Asterisk (*) indicates temperature recorded from the University of Minnesota St. Paul Climate Observatory; ND indicates no data recorded. University of Minnesota St. Paul campus dairy, spring 2017 to fall 2018.

Date	Air (°C)	Soil (°C)	Internal 1 (°C)	Internal 2 (°C)
8 May 2017	17.9	21.5	28.2	27.5
9 May 2017	16.7	17.7	19.6	17.6
10 May 2017	16.3	15.5	16.2	15.9
11 May 2017	16.5	15.6	18.3	17.1
12 May 2017	18.7	16.6	20.1	19.9
13 May 2017	21.7	19.2	24.3	22.1
14 May 2017	22.1	20.6	27.3	23.5
15 May 2017	18.5	21.5	28.6	22.9
16 May 2017	21.0	21.7	28.9	22.7
17 May 2017	17.5	21.5	26.8	22.6
18 May 2017	10.8	17.2	17.5	15.3
19 May 2017	9.3	15.5	18.6	13.3
20 May 2017	7.6	13.8	14.3	11.5
21 May 2017	9.2	12.7	14.5	11.9
22 May 2017	14.3	15.0	23.8	17.7
23 May 2017	13.4	19.4	31.1	22.5
24 May 2017	13.1	20.4	29.5	26.2
25 May 2017	16.8	25.7	26.7	36.6
26 May 2017	20.9	29.5	24.9	39.0
27 May 2017	19.1	27.6	19.8	39.2
28 May 2017	18.0	20.9	18.2	21.2
29 May 2017	14.9	16.9	14.8	15.1
30 May 2017	13.1	14.9	13.3	13.6
31 May 2017	18.5	16.3	17.8	18.7
1 June 2017	20.7	18.4	18.7	22.8

2 June 2017	24.7	20.6	22.7	25.9
3 June 2017	27.2	23.3	25.5	28.4
4 June 2017	26.2	23.2	24.9	27.8
5 June 2017	23.3	26.3	26.0	26.4
9 Jun 2017	29.9	29.7	35.6	38.1
10 Jun 2017	28.9	26.4	32.7	28.8
11 Jun 2017	20.6	24.4	26.0	22.6
12 Jun 2017	22.2	24.8	27.3	28.8
13 Jun 2017	25.4	26.2	32.9	35.3
14 Jun 2017	24.4	31.6	39.0	35.0
15 Jun 2017	25.1	34.1	38.6	27.2
16 Jun 2017	23.6	32.7	31.2	24.4
17 Jun 2017	23.1	29.7	27.0	24.9
18 Jun 2017	19.8	29.0	22.3	20.2
19 Jun 2017	20.6	27.1	22.3	21.3
20 Jun 2017	21.6	25.2	26.2	21.2
21 Jun 2017	22.5	25.9	28.6	23.8
22 Jun 2017	19.8	23.8	22.8	20.7
23 Jun 2017	19.0	21.4	20.7	17.6
24 Jun 2017	16.5	20.3	17.8	16.3
25 Jun 2017	16.4	18.1	17.0	16.8
26 Jun 2017	12.6	16.6	12.9	12.3
27 Jun 2017	15.6*	ND	ND	ND
28 Jun 2017	18.6*	ND	ND	ND
29 Jun 2017	21.1*	ND	ND	ND
30 Jun 2017	18.9*	ND	ND	ND
1 Jul 2017	20.3*	ND	ND	ND
2 Jul 2017	20.3*	ND	ND	ND
3 Jul 2017	19.7*	ND	ND	ND
13 July 2017	20.8*	ND	ND	ND

14 July 2017	24.7	26.2	33.2	33.6
15 July 2017	26.7	26.0	29.2	28.2
16 July 2017	26.1	27.3	33.0	29.3
17 July 2017	26.6	30.0	38.5	30.1
18 July 2017	23.4	30.3	35.9	34.6
19 July 2017	21.9	32.3	34.8	37.6
20 July 2017	26.4	34.9	37.0	32.2
21 July 2017	24.1	35.3	26.9	26.3
22 July 2017	27.6	33.6	30.9	29.6
23 July 2017	23.2	28.4	24.6	24.5
24 July 2017	21.8	25.8	26.6	24.7
25 July 2017	22.8	25.4	26.4	24.6
9 Aug 2017	21.1*	ND	ND	ND
10 Aug 2017	17.2*	ND	ND	ND
11 Aug 2017	17.2*	ND	ND	ND
12 Aug 2017	18.1*	ND	ND	ND
13 Aug 2017	19.4*	ND	ND	ND
14 Aug 2017	17.5*	ND	ND	ND
15 Aug 2017	17.2*	ND	ND	ND
16 Aug 2017	19.4*	ND	ND	ND
17 Aug 2017	20.8*	ND	ND	ND
18 Aug 2017	17.8*	ND	ND	ND
19 Aug 2017	17.5*	ND	ND	ND
20 Aug 2017	20.3*	ND	ND	ND
21 Aug 2017	22.5*	ND	ND	ND
22 Aug 2017	20.0*	ND	ND	ND
23 Aug 2017	16.9*	ND	ND	ND
24 Aug 2017	16.9*	ND	ND	ND
25 Aug 2017	17.2*	ND	ND	ND
26 Aug 2017	16.4*	ND	ND	ND

27 Aug 2017	16.7*	ND	ND	ND
28 Aug 2017	18.3*	ND	ND	ND
29 Aug 2017	16.9*	ND	ND	ND
30 Aug 2017	19.7*	ND	ND	ND
31 Aug 2017	18.9*	ND	ND	ND
1 Sept 2017	15.8*	ND	ND	ND
2 Sept 2017	16.4*	ND	ND	ND
3 Sept 2017	19.7*	ND	ND	ND
4 Sept 2017	21.7*	ND	ND	ND
5 Sept 2017	16.4*	ND	ND	ND
6 Sept 2017	12.2*	ND	ND	ND
8 Sept 2017	18.4	19.0	31.6	29.9
9 Sept 2017	18.3	18.7	22.0	22.2
10 Sept 2017	20.9	20.0	22.4	22.7
11 Sept 2017	22.1	20.5	23.8	23.0
12 Sept 2017	24.0	21.6	29.8	22.9
13 Sept 2017	24.9	23.0	37.4	27.7
14 Sept 2017	25.8	26.0	37.3	31.1
15 Sept 2017	25.4	27.8	34.0	35.7
16 Sept 2017	22.4	26.3	24.0	36.8
17 Sept 2017	15.8	23.0	17.1	33.3
18 Sept 2017	12.8	19.3	15.9	20.1
19 Sept 2017	20.8	21.1	20.6	23.6
20 Sept 2017	21.8	22.4	21.1	24.4
21 Sept 2017	21.0	21.4	23.1	23.2
22 Sept 2017	28.5	25.0	27.2	27.6
23 Sept 2017	27.8	25.9	27.9	28.5
24 Sept 2017	26.6	25.3	26.9	27.2
25 Sept 2017	16.6	21.5	19.0	21.0
26 Sept 2017	15.1	18.7	17.0	18.2

27 Sept 2017	14.4	16.9	15.8	16.8
28 Sept 2017	16.4	17.0	16.9	17.4
29 Sept 2017	14.6	16.7	15.5	16.6
30 Sept 2017	15.0	16.1	15.2	16.0
1 Oct 2017	14.6	15.6	14.0	15.2
2 Oct 2017	16.3	15.7	15.7	15.9
3 Oct 2017	16.8	17.3	16.9	17.8
4 Oct 2017	12.2	14.6	13.2	14.5
5 Oct 2017	13.3	14.4	14.2	15.0
6 Oct 2017	12.2	14.3	13.9	14.8
7 Oct 2017	14.5	14.8	14.4	15.5
8 Oct 2017	16.6	15.2	15.1	15.9
9 Oct 2017	10.0	12.5	10.5	11.9
12 Oct 2017	14.1	14.6	19.3	20.9
13 Oct 2017	12.3	13.4	13.3	14.4
14 Oct 2017	9.5	11.5	10.6	10.9
15 Oct 2017	11.8	10.5	10.1	10.3
16 Oct 2017	11.8	10.2	10.3	10.6
17 Oct 2017	15.6	11.4	12.8	13.1
18 Oct 2017	15.5	12.4	13.7	14.2
19 Oct 2017	15.3	11.5	12.2	12.6
20 Oct 2017	19.1	13.0	15.2	15.0
21 Oct 2017	16.5	14.7	16.1	16.1
22 Oct 2017	13.1	13.0	13.4	14.0
23 Oct 2017	11.5	11.8	12.3	12.8
24 Oct 2017	8.0	9.5	9.3	10.0
25 Oct 2017	9.7	8.6	8.7	8.9
26 Oct 2017	7.4	9.0	9.3	9.2
27 Oct 2017	0.8	6.0	4.0	4.5
28 Oct 2017	-0.5	4.0	1.9	2.0

29 Oct 2017	2.1	3.6	2.2	1.8
30 Oct 2017	2.0	4.1	3.2	3.2
31 Oct 2017	-0.6	2.7	1.5	1.8
1 Nov 2017	-0.2	2.1	0.5	0.6
2 Nov 2017	2.5	2.9	2.5	2.0
3 Nov 2017	-0.6	2.5	1.3	1.4
4 Nov 2017	1.2	2.4	1.4	1.2
2 Dec 2017	3.3*	ND	ND	ND
27 Apr 2018	18.4	19.3	27.7	26.1
28 Apr 2018	10.6	10.7	13.8	14.4
29 Apr 2018	13.7	10.6	13.4	13.7
30 Apr 2018	19.4	12.1	15.0	16.3
1 May 2018	19.8	14.9	19.2	20.6
2 May 2018	15.9	15.1	18.6	18.8
3 May 2018	19.5	15.3	19.6	19.4
4 May 2018	20.8	15.7	21.4	20.4
5 May 2018	20.6	16.2	21.9	20.9
6 May 2018	19.7	17.0	23.8	21.4
7 May 2018	23.6	19.8	32.4	26.4
8 May 2018	18.0	23.7	26.1	29.7
9 May 2018	14.9	20.6	23.3	20.3
10 May 2018	13.5	21.8	23.3	22.8
11 May 2018	9.8	15.6	14.0	18.0
12 May 2018	12.0	14.8	15.0	15.5
13 May 2018	16.6	19.1	20.3	19.0
14 May 2018	18.6	19.3	22.3	20.0
15 May 2018	19.7	21.2	21.9	20.9
16 May 2018	23.3	23.9	25.2	22.8
17 May 2018	23.5	24.1	26.0	25.0
18 May 2018	22.3	22.5	24.7	23.7

19 May 2018	15.9	17.8	21.3	16.7
20 May 2018	15.8	16.7	18.4	17.3
21 May 2018	18.0	18.4	20.6	18.8
22 May 2018	20.0	19.3	22.2	20.7
23 May 2018	23.3	20.7	23.7	23.8
24 May 2018	26.6	22.6	26.8	28.3
25 May 2018	25.3	24.2	25.0	26.1
26 May 2018	28.1	25.7	27.7	29.2
27 May 2018	28.0	24.3	26.7	26.7
28 May 2018	30.0	26.5	28.5	31.0
29 May 2018	25.1	25.0	25.3	26.6
4 Jun 2018	22.3	21.6	32.2	25.6
5 Jun 2018	22.0	22.5	26.2	26.1
6 Jun 2018	22.5	22.0	24.7	24.3
7 Jun 2018	21.4	21.2	27.2	24.2
8 Jun 2018	20.6	22.1	31.4	26.4
9 Jun 2018	19.6	22.6	31.9	28.5
10 Jun 2018	21.4	25.9	30.4	34.8
11 Jun 2018	19.5	28.4	20.9	34.3
12 Jun 2018	23.1	32.1	25.9	32.2
13 Jun 2018	22.5	31.7	25.9	26.9
14 Jun 2018	23.2	27.2	26.0	27.5
15 Jun 2018	26.8	26.1	28.3	28.7
16 Jun 2018	25.3	25.5	27.4	25.4
17 Jun 2018	28.1	28.8	30.5	28.5
18 Jun 2018	22.2	25.3	26.6	24.6
19 Jun 2018	18.2	22.4	22.1	21.2
20 Jun 2018	22.9	23.1	24.6	24.8
21 Jun 2018	22.6	23.9	24.9	24.7

9 Jul 2018	28.0	26.6	27.2	ND
10 Jul 2018	24.9	26.6	25.1	ND
11 Jul 2018	26.9	26.2	27.2	35.9
12 Jul 2018	27.3	28.6	32.2	37.2
13 Jul 2018	24.2	30.3	36.6	34.8
14 Jul 2018	27.6	35.3	39.9	36.5
15 Jul 2018	26.7	38.8	36.2	36.1
16 Jul 2018	24.9	33.3	28.8	32.2
1 Aug 2018	20.6	24.8	26.7	25.5
2 Aug 2018	19.0	22.8	23.4	22.8
3 Aug 2018	22.0	22.6	23.7	23.9
4 Aug 2018	22.3	22.9	23.3	30.8
5 Aug 2018	25.6	24.7	26.2	38.0
6 Aug 2018	24.4	27.1	32.0	34.2
7 Aug 2018	24.3	32.8	29.7	28.0
8 Aug 2018	25.7	37.2	31.6	33.0
9 Aug 2018	27.8	35.5	32.2	33.3
10 Aug 2018	27.2	30.5	31.2	31.2
14 Sept 2018	25.7	24.2	28.8	27.9
15 Sept 2018	28.3	25.1	27.0	29.1
16 Sept 2018	27.6	26.1	28.1	36.2
17 Sept 2018	21.6	27.6	26.0	33.4
18 Sept 2018	16.3	24.9	21.1	30.9
19 Sept 2018	15.7	24.4	21.3	20.7
20 Sept 2018	16.3	22.2	18.7	18.5
21 Sept 2018	12.8	19.2	15.2	13.8
22 Sept 2018	13.1	18.4	14.6	12.9
23 Sept 2018	18.0	22.0	18.5	18.3
24 Sept 2018	18.8	21.5	18.9	19.4